seem to have a case in point of the prevalence of particular species of shells being influenced by wave-currents acting on sea-bottoms that do not remain constant in constitution and character.

Preliminary Account of the Development of the Lesser Weever-Fish, *Trachinus vipera*. By George Brook, F.L.S.

(Read 1st May, 1884.)

[Plates III.–VI.]

The observations on which my paper is based have been made on eggs laid in my aquarium by fish which I have had in the tanks over two years. The conditions under which the development was carried on will not therefore be normal, and the direct rays of the sun were never allowed to fall on the eggs, as would be the case in nature.

The eggs of *Trachinus vipera* are laid in the summer. I have had them as early as April, both last year and this, as floating eggs. Dead eggs have been found at the bottom of the tanks in March. The eggs found in April were very few, and often not fertilized. It was not till the 6th of June that they began to show in any numbers, and with but a small proportion of unfertilized ones. They continued to be laid at intervals of three or four days during June and July; but the batches laid during the last few days of July were again few in number, and with a large proportion of unfertilized ova amongst them; and no ova were found in August at all.

The egg of *Trachinus vipera* is about 1·32 millim. in diameter, of a beautiful pearly white, and quite translucent, and contains from 20 to 30 small oil-globules which cause it to float on the surface of the water. These oil-globules are scattered over the upper hemisphere of the yolk, and lie between it and the vitelline membrane. They vary in size from ·12 to ·03 millim. The oil-globules cause the egg to float with the germinal disk downwards, so that the embryo is developed on its back, so to speak, and it is not until some time after hatching that the young fish is enabled to swim with the ventral surface downwards.

Eggs freshly extruded from the ovary are not spherical, as the egg-membranes are larger than the yolk, and appear wrinkled until the "breathing-cavity" gets filled with water, and it is

then that the egg rises to the surface. Although the number and size of the oil-globules is variable, this is only within certain limits; and I have never found an egg of *Trachinus* with only one large oil-globule, as appears to be the rule with the majority of other species of pelagic eggs hitherto described.

The eggs are laid in the night; but at what time I am not certain. We have watched the fish up to 1 A.M., and resumed watch again as early as 5 A.M., but have never been able to catch them in the act of ovipositing. They are probably laid in the very early hours of the morning, just before or after daybreak, as we always found them well advanced in the segmentation stage by even 5 A.M. I have this year, however, had an opportunity of studying the segmentation process from its commencement, in a few eggs laid in the beginning of April and at a temperature 9 or 10 degrees lower than at their normal time of appearing last year, and consequently considerably retarded in development. The eggs, being at the surface of the water, are naturally more affected by the temperature than would be the case otherwise; so that in order to estimate fairly the rate of development the temperature of both room and water must be taken into account. The temperature of the water in my aquarium varied during the months of June and July from 54 to 58 and 60, and that of the room from 54 to 62, and during the last two days it went up to 65.

A comparison of the various times at which the embryo developed certain organs or structures made in various batches of eggs at various temperatures, showed that a difference of about 2 degrees would retard or accelerate from 9 to 12 hours in the early stages, and a whole day for hatching. I propose, however, to leave the consideration of this part of my subject to a future paper.

Egg-Membranes.—In the fertilized floating ovum the following investing membranes can be distinguished :—

(1) An exceedingly thin membrane showing only as a fine line under the 1-inch objective, hyaline and apparently structureless and non-perforate. This is the *vitelline membrane* according to the definition of Balfour.

(2) Within this, and occasionally separated from it by a space, is a much thicker membrane, the *zona radiata*; but I have made no observations on its structure. This is separated from the yolk by a space, the "breathing-chamber" of Ransom, which is only small in the species under discussion. Ryder describes only

one membrane (the *zona radiata*) in the Spanish Mackerel, as also Messrs. Kingsley and Conn in the Cunner. The outer membrane may indeed be only an outer layer of the *zona radiata*; but it is easily separated and made prominent by the use of reagents. Balfour ('Comp. Embryol.' i. p. 50) says that "in osseous fishes the vitelline membrane is usually either absent, or may perhaps in some instances, *e. g.* in the Perch, be imperfectly represented. In the ripe ovum of the Herring there is a distinctly developed membrane exterior to the *zona radiata*, which is probably the vitelline membrane." A vitelline membrane does not, however, appear to have been usually recognized in pelagic teleostean eggs.

The oil-globules scattered over the upper hemisphere are situated inwardly to the *zona radiata*, and sink into little pockets pushed into the yolk from its surface. These two membranes may also be distinctly seen in the unfertilized egg ready for extrusion taken from the body of the fish; but they are then in a relaxed and shrivelled condition. After the closure of the blastoderm at the caudal end of the embryo, the yolk becomes invested by another membrane, which is termed by Ryder the "epiblastic sac," the origin and development of which will be discussed in its proper place.

The *zona radiata* appears to become thinner as development proceeds.

Micropyle.—I have several times seen the circular micropyle opening in the *zona radiata*. It seems to be surrounded by a depression on the outer surface of that membrane, causing a slight protuberance on the inner surface.

Sect. 1. *Segmentation Stage to Formation of Blastodermic Rim.*

9 A.M. In the newly laid ovum the germinal disk is not distinguishable until the first furrow begins to form, faint at first, then forked at each end (fig. 1); and the forking may be seen gradually creeping round until the first two cells are formed, as shown in fig. 2. This takes about an hour to accomplish; and the first furrow is then very thick and distinct, the outline of the cells gradually shading off as it recedes from the furrow until on the opposite side it is only barely visible. Somewhat later this outline becomes more sharply defined. When first formed the two cells of the disk are about ·35 millim. in diameter each; but these increase in size to ·41 millim. before any further subdivision

takes place. The nuclei in the first two cells could not be distinctly made out; but at about 11.15 A.M. a second furrow (fig. 3) began to appear at right angles to the first. Each furrow begins in the centre and extends outwards, deepening more rapidly at its origin, so that the furrow is complete at the inner margin of the cell before it has reached the outer (fig. 5). When the furrow reaches (about 11.45 A.M.) the outer margin, the latter becomes indented, and the four-celled stage is then fully marked out as shown in fig. 4; and two minutes later nuclei appear, but are only faintly visible in this stage. At 12.50 P.M. the nuclei had completely disappeared, and the blastodisk had become almost square. At 1.10 the segmentation-furrows were beginning to form in the same general direction as the first furrow, but somewhat at an angle with it (fig. 5). At 1.35 the eight-celled stage was completed, as in fig. 6, with the nuclei again visible. By 2.30 the nuclei had again completely disappeared; and at 2.40 new furrows made their appearance at right angles to the previous (fig. 6). At 2.55 the sixteen-cell stage was complete, but the nuclei did not appear until ten minutes later, and in this stage are very distinct (fig. 7). The nuclei had again disappeared at 3.35; and from this time the segmentation is not quite regular. The first cells to divide are the two centre ones on each side, next the corner cells, and afterwards the four central cells; but the whole process only occupies 15 to 20 minutes, and this time the four central cells no longer divide vertically, but in a horizontal plane so as to form two layers. From this point it is difficult to follow the process of segmentation, the outlines of the cells being too confused through overlapping. The disk is now almost circular, and has a diameter of about ·73 millim. Two hours later segmentation had progressed rapidly, particularly in the centre, where the cells were at least four rows deep. About this time free nuclei make their appearance in the "nuclear zone," and free cell-formation takes place. In two hours' time a first row of these free cells has been formed round the disk, and there are a few isolated cells of a second row (fig. 8). A little later a partial side view of the egg showed that the cells of the "nuclear zone" had pushed their way partly under the outer edge of the blastodisk. Three hours later it is found that segmentation has proceeded; but there is still no increase in the diameter of the blastodisk, which is now quite circular. The disk has now the appearance presented in fig. 9. There are three

rows of nuclei around the disk; but the outline of cells cannot be made out. These nuclei in the majority of ova are fairly uniformly distributed, though not with the geometric precision of Kupffer's figure ("Beobacht. ü. d. Entw. d. Knochenfische," Schultze's Archiv, Bd. iv. Taf. xvi. fig. 1). Later on considerable irregularity begins to show itself, which will be referred to in its proper place. The blastodisk at this stage is almost flat on the under surface, or perhaps very slightly convex, and has perceptibly increased in thickness at the centre. There is no material change in the blastodisk 12 hours later; the "nuclear zone," however, has altered considerably. The zone has increased in width, the nuclei (which are very distinct) have increased considerably in number, and are now most irregular in their arrangement; so much so that it is impossible to distinguish a series. The majority of nuclei are in pairs; but here and there are clusters of three and four, in some cases four arranged in a chain. This is possibly a consequence of the subdivision of the nuclei. About 30 hours after the first furrow is formed the blastodisk begins to extend over the yolk; it also becomes thinner at the centre, and is now concave on the under surface. No trace of the segmentation-cavity is yet to be found, nor does there appear to be that stratification in the cells which, as described by Messrs. Kingsley and Conn, is the case in the embryo of the Cunner. About $4\frac{1}{2}$ hours later the first beginning was noticed of the rim which ultimately forms the boundary of the segmentation-cavity. It showed itself on a surface view of the underside of the blastodisk as a short line rising a little within its margin, this line gradually extending itself parallel with the blastodisk margin in each direction until about 3 hours later the outline was sharply and definitely marked out all round. The blastodermic ring thus formed is a little broader at one point whence the future embryo will develop (fig. 10). Diameter at this stage ·88 millim. No separation of the subjacent cells from the blastodisk to form the segmentation-cavity has yet taken place, and its origin will be considered in the next section.

The so-called invagination of the hypoblast is very clearly made out, in optical section, as at first a single layer of cells sharply defined in the upper and lower limits of layer (fig. 11). The origin of this layer in teleostean fishes is not clearly understood. According to most recent investigations, Henneguy (Comptes Rendus, xcv. 1882, pp. 1297–1299) maintains that this

invaginated hypoblast arises in the Trout by an involution of the nervous layer of the epiblast, and that the epidermic layer ends on the surface of the yolk, taking no part in the process. On the other hand, Messrs. Kingsley and Conn maintain that in the Cunner the "epidermic layer" only of the epiblast takes part in the invagination. According to Balfour, in smaller teleostean eggs the nucleated cells of the intermediary layer form the hypoblast. Without the aid of sections it is impossible to tell positively what really does take place; and the difficulty of preserving and hardening pelagic eggs in this stage is well known. I am, however, inclined to think that Henneguy is right, and that the invagination observed in optic section in the living egg is an inward folding of the lower layer cells of the epiblast. The cells thus formed are, to begin with, in a single layer only, the outline of which, top and bottom, is very well marked, but the cells themselves are not columnar as described by Kingsley.

Intermediary Layer=Parablast of Klein.—My observations confirm Van Bambeke's and Klein's figures and descriptions as regards the presence of a thickened peripheral layer or welt, the "nuclear zone" of Kupffer. This thins off under the blastodisk, and also gradually becomes indistinguishable as it passes away from the blastodisk round the yolk. Very early in the segmentation of the disk, certainly as early as the eight-cell stage, and probably earlier, there is visible around the disk a granular zone, the largest granules being nearest to the disk, and becoming finer and finer further away. This is a prominent zone in the sixteen-cell stage (fig. 7), and it is in this zone that the free cell-formation already described takes place. A cellular structure has certainly been traced under the rim of the blastoderm, but not far, probably only so far as the thickened portion of the blastodermic rim extends. There is, however, nothing in the intermediary layer under the disk corresponding to the median lens (*lentille*) described by Van Beneden.

Retardation of this Stage.—The development described up to the present was observed in April at a mean temperature of 49° F.; whereas the later stages were observed in July at a mean temperature of 60° F. Thus the rate of development in this section is altogether out of proportion to the remainder; but I have had to avail myself of these earlier observations, because in the hot summer weather the eggs laid during the night were well advanced in the segmentation stage by 7 A.M.; and thus I

have no record of the early segmentation at higher temperatures. The difference is somewhat as follows:—Whereas at 49° F. it took 2½ days for an egg to advance from stage of fig. 9 to that of fig. 12, at 60° F. this was accomplished in 22 hours.

Sect. 2. *From the first Formation of the Embryo to the Closure of the Blastopore.*

The embryonic area encroaches considerably further on the segmentation-cavity than is shown in fig. 10 before the first traces of the embryo make their appearance. Shortly before this takes place semidetached and quite detached cells make their appearance about the apex of the embryonal shield. These cells rarely have a rounded shape, but are mostly more or less angular, and often have a pointed prolongation. These detached cells, so far as I could make out, are lying loose on the floor of the segmentation-cavity. This, however, is here so shallow that they appear to fill up the whole area in an "optical section," and cannot be made out clearly in this way. Can these be cells pushed up from the intermediary layer to take part in the formation of the alimentary tract, as suggested by Kingsley is the ultimate purpose of the intermediary layer? or are they detached cells from the invaginated hypoblast or lower layer cells? As the embryonic shield encroaches on the segmentation-cavity, the latter becomes shallower as well, until after the embryo forms and the blastoderm extends still further it becomes, as at first, a mere fissure of separation between the blastoderm and the intermediary layer. Ryder ('Bull. U.S. Fish. Comm.' i. p. 147), quoting from an earlier paper in 'Forest and Stream,' asserts that the segmentation-cavity does not disappear at a very early stage of embryonic life, as Balfour and others supposed, but that it "is filled with fluid, and grows with the growth of the germinal disk, as the latter becomes converted into the blastoderm, and does not disappear until some time after the embryo has left the egg as a young fish, after remaining as a space around the yelk-sac as long as a vestige of the latter remains." My observations appear to confirm the view taken by Ryder. An optical section made after the blastoderm has already crossed the equator of the egg is given in fig. 14, where it will be seen a double line runs forward from the head end of the embryo to the thickened rim, enclosing a cavity which Ryder says is filled with fluid. This double line, with its enclosed cavity, follows the course of the blastodermic

rim as it extends over the yolk, until the latter is completely enclosed. The application of reagents, by bringing about a shrinking of the yolk, causes the outer membrane to separate further from the inner or intermediary layer which remains attached to the yolk. This shows that a fissure still exists between the two layers; indeed it can be traced all through the embryonic life so long as the yolk-sac persists. The distance between the two membranes of this cavity becomes more pronounced in the newly hatched embryo (fig. 27). I have also, as shown in fig. 14, traced a prolongation of the segmentation-cavity beneath the fore part of the head; but have not satisfied myself that it is pushed forward to form the pericardiac cavity, as is maintained by Ryder; although later stages seem to support this view. If the cavity really does extend so far, the line formed by its two membranes under the hind portion of the head is so minute in the living egg as to look single only. In the newly hatched embryo, however, part of the yolk has been absorbed, and the heart then shows clearly in the same cavity as is continued around the yolk.

Kingsley and Conn, in the text of their paper, combat Ryder's views on this point altogether, but add, in a footnote, that later studies make them more inclined to accept them, at any rate partially.

The first step towards the formation of the embryo is the appearance of a faint streak, the keel, in the median line of the anterior portion of the embryonal shield. This keel lengthens, and becomes club-shaped as the embryonal area encroaches on the segmentation-cavity. As the development advances, the anterior portion of the keel is seen to widen, and the outline becomes spathulate. This is caused by outgrowths springing from the keel, one on each side, to form the optic lobes. At first these only appear as slight swellings, but soon a curved line makes its appearance on each side, marking the angle formed by the optic lobes with the keel. By this time the growing blastoderm has already passed the equator of the egg. The optic lobes begin to be segmented off before the protovertebræ form, and the process is completed about the time four or five protovertebræ are visible. The lumen begins to show before eight protovertebræ are formed, and before the blastodermic ring has closed.

The division of the fore part of the medullary cord into fore,

mid and hind brain shows itself before the optic lobes are segmented off, and is well marked at the stage of four or five protovertebræ. The fore brain seems to be marked off from the posterior portion first, then the posterior portion is afterwards divided into mid and hind brain. About the time that the optic lobes are fully segmented off, a lumen appears in the mid brain and almost simultaneously one in the fore brain also, while that in the hind brain develops more slowly, not appearing till 8 protovertebræ are formed, or even later.

The exact time at which the notochord appears was not noted, but it was already a well-marked feature by the time the optic lobes were outwardly marked out from the keel, when it is to be seen extending well up to the eye-lobes. Posteriorly it widens out, and its cells seem to merge insensibly with those of the surrounding tissue; at least, I have not been able to trace it quite up to the edge of the blastoderm.

Kingsley and Conn state that the notochord originates in the hypoblast, that it is then pushed up through the mesoblast, dividing the latter into two lateral plates. I have not been able to verify this statement up to the present. When first observed, the notochord appeared rather flattened in transverse section, and the lateral mesoblastic plates quite distinct.

With the separation of the optic lobes, the two tracts of mesoblast begin to be divided into somites. The first traces of protovertebræ were observed after about sixty hours' development, and after the formation of Kupffer's vesicle.

The thickening of the epiblast forming an invagination for the eye-lens has been observed as early as in embryos with three protovertebræ, but more generally when the embryo possesses about six protovertebræ. My observations of its further development and the separation of the lens were but a confirmation of what is already known on the subject.

It was difficult to make out the auditory vesicles during the process of invagination, and one only began to recognize them clearly by the time the process was completed. Invagination seems to begin, however, very soon after that for the optic lenses, and they are fully marked off, as shown in fig. 19, by about the stage of eight protovertebræ. The lenticular body has an amber tint. About the time that the eye-lenses first show traces of invagination, a broadening out of the tissue on each side of the hind brain is seen to begin; the embryonic border (Embryonal-

saum of Kupffer), which lies outside this, again widens also, and often assumes a pointed form. Probably this widening is the result of the invagination to form the auditory sacs; but I could not make out clearly what was going on until the process was completed. The nasal pits appear also to originate about this time.

Kupffer's vesicle (postanal vesicle of Balfour) appears shortly after the optic lobes are formed, soon after the growing blastoderm has passed the equator of the egg, and therefore a considerable time before the closure of the blastopore. I have not been able to detect the slightest relation between it and this closure, although it certainly increases rapidly in size at the period when the rim is nearly closed, and attains its maximum development soon after the closure. It arises before any protovertebræ are formed, and at the time of its disappearance there are seventeen or eighteen somites. Its proximate origin is signalized, as Kingsley and Conn state, by the appearance of a few granules which draw together, and shortly afterwards the vesicle is seen on optical transverse section as a very flattened lenticular body, amber-tinted, and which, at its first appearance, seems solid. Kingsley states that Kupffer's vesicle arises in the Cunner when the blastoderm has covered over three quarters of the yolk, and after many protovertebræ have been formed. This is consequently at a much later stage in *Trachinus*. Ryder has, in different species, noticed the first appearance of this vesicle at periods varying from the time when the blastoderm covers three quarters of the yolk up to nearly when the closure of the blastopore takes place. In the Trout it appears when the blastoderm has just passed the equator, and in the Perch it does not appear until after the closure of the blastopore.

About, or shortly after, the formation of protovertebræ, free pigment-spots make their appearance, scattered irregularly over the embryo. These increase in number and size until, at about the time the heart begins to pulsate, they assume a stellate form. They do not seem, however, to develop exactly *pari passu* with the embryo; at times they are slightly accelerated or retarded. The assumption of the stellate form may also be either before or after the heart begins to pulsate. Kingsley found that in the Cunner they arise at the same time as the protovertebræ.

The expanding blastoderm continues to grow over the yolk, until gradually it leaves a mere pore enclosed by the thickened

rim of the blastoderm, the axis of the embryo terminating posteriorly on its anterior edge. This pore is ultimately seen to close up, or at least apparently, and nothing left but a thickened patch. This blastodermic pad is, as may be seen from the account of its formation, composed of all that remains of the thickened rim of the blastoderm after it has encircled the yolk, and will, as pointed out by Kupffer, His, and Ryder, enter largely into the formation of the tail, which we shall presently see sprout out in continuation with the axis of the embryo. As Ryder points out, this blastodermic pad may be considered a true tail-swelling. At the time of closure of the blastopore nine or ten protovertebræ are formed, and the tail end of the embryo is somewhat spathulate in shape. The widened part includes the notochord, with an unsegmented plate of mesoblast on each side. It is still impossible to trace the notochord to the extreme posterior edge of the embryo.

We have now brought our consideration of the development of the embryo up to a stage when there are nine or ten protovertebræ. The invagination of the eye-lenses has progressed but slowly. The auditory vesicles, however, have been fully closed in, and the nasal pits are formed. The brain is now divided into the three embryonic regions, and a lumen has appeared in all three, although one does not show itself in the spinal cord until later.

The optic bulbs have become fully differentiated and the lumen has appeared.

Kupffer's vesicle has nearly reached its maximum development and the pigment-spots are well established.

Sect. 3. *From Closure of the Blastopore to the Pulsation of the Heart.*

The first important developments after the closure of the blastopore are the formation of the heart and of the alimentary canal, and both seem to arise at the same time, on the closure of the pore or immediately after it. The heart begins as a small patch often distinguishable by its light amber colour. The patch is situated a little to the right of the central line of the embryo, as seen ventrally, and almost beneath the hind brain, and was most readily noticed on a side view. It has been found as early as the stage when eight protovertebræ are formed (the blastopore being closed), but is usually first distinguishable at a stage when there

are eleven or twelve protovertebræ. In the side view it is seen as a thickening beneath the embryo (see fig. 20). I found it difficult to get a clear view of the changes taking place in this patch in the living egg, and can add nothing from this point of view to its development. Many of the cells are, however, so far as I can make out, budded off from the mesoblastic roof of the cardiac cavity, just as Ryder describes, and then grow down and come in contact with the floor of the cavity. Messrs. Kingsley and Conn observed the same process of development in the Cunner. The heart is solid at first, but a lumen afterwards develops when it has reached the floor of the pericardiac space. The heart is only a simple hollow cellular tube at the time it commences pulsating, and has its broad venous end closely applied to the vitellus. The first pulsations, faint and somewhat intermittent, were observed at the stage of about twenty protovertebræ, and about the same time the first spontaneous movements of the embryo have been noticed. The venous end of the heart is somewhat funnel-shaped, and remains applied to the vitellus up to the time of hatching. No blood-corpuscles nor circulation is visible up to three or four days after hatching. The observations of Ryder and Kingsley and Conn seem to agree with my own on this point—no vascular system has been found up to a considerable time after hatching. Ryder found that in the Spanish Mackerel the aorta only begins to develop 16–20 hours after hatching, the whole development up to hatching only occupying 24 hours. I therefore conclude that I have not been able to keep my embryos long enough to follow the development of the vascular system. This seems in strange contrast with observations on non-pelagic eggs, which usually show a very marked circulation, both in the embryo and around the vitellus, a considerable time before hatching.

Intestine.—On the closure of the blastopore, and when Kupffer's vesicle is at its maximum development, a thin layer of granules extends from the vesicle to the blastopore, which is either the homologue of the postanal gut or of the neurenteric canal. If Balfour is correct in identifying Kupffer's vesicle with postanal vesicle of Elasmobranchs, this layer of granules should represent the first formation of the neurenteric canal, and the anus should be formed at a point anterior to the vesicle. The mesenteron is, however, developed from the granular band forwards, and after

Kupffer's vesicle has atrophied the anus seems to be formed pretty nearly at the same point; but I have not been able to make out clearly the relationship between Kupffer's vesicle and the mesenteron.

General advance.—At the time of closure of the blastopore the brain was already marked out into its three regions, and a lumen had developed in them. Shortly afterwards a lumen is seen extending through the whole length of the cerebro-spinal cord, and before the heart has begun to pulsate the hind brain has been further differentiated, in that the cerebellum has been fully separated off from the medulla oblongata. The nasal pits are now large and well defined. The inner lens of the auditory capsule has increased in size, become hollow, and simultaneously with the pulsation of the heart the otoliths make their appearance. The lenses have become fully separated from the optic bulbs. The pigment-spots have assumed a stellate form. The first rudiments of lobes to form pectoral fins may be found just before the heart begins to pulsate. Generally also the tail has commenced its growth, and the first folding of the epiblast to form the caudal fin is an accompanying phenomenon.

Sect. 4. *From the Pulsation of the Heart to the time of Hatching.*

Liver and Pancreas.—As the mesenteron increases in size a lumen arises throughout its course and extends quite into the head. At the beginning of the sixth day of development a ventral swelling makes its appearance in that part of the mesenteron lying between the rudimentary pectoral fins—the first rudiment of the liver. This becomes more marked as the tail curves round the yolk, and about the end of the seventh or beginning of the eighth day, the liver presents a lobulated character. Before this time, however, another organ, the *pancreas* (fig. 23), makes its appearance on the dorsal side of the intestine, and slightly posterior to the origin of the liver. It appears soon after the liver arises, and towards the end of the sixth day, as a pocket pushed out from the mesenteron. The mesenteron, up to period of outgrowth of the liver and pancreas, was straight, but increasing size of these two organs, as well as of the mesenteron itself, causes the canal to take an S-shaped form, and this becomes still more involved as development proceeds.

The *proctodeum* seems to arise rather late in development. I

have not been able to trace it through all its phases. A strand of cells extends from the point where the future anus will open, upwards and inwards to meet the mesenteron, and the proctodeum is probably invaginated along this short strand of cells, but no lumen is formed up to at least four days after hatching. The constriction in the alimentary canal which is shown in figs. 27 & 29 is not, as might be at first supposed, evidence of the junction of the proctodeum and the mesenteron. It is in reality a fold in the mesenteron itself produced by a bending down of its apex in consequence of the development of the embryo, and is more marked in some individuals than in others. It perhaps may be said to mark off the cloacal part of the mesenteron. The mesenteron, which has widened out up to the point where the liver arises, suddenly narrows, as seen in side view, and extends as a comparatively narrow tube into the region of the head. The changes which take place in the respiratory section of the mesenteron are exceedingly difficult to follow in the living embryo, owing to its being curled up within the shell, and I propose to leave a discussion of this portion until I have made an investigation of stained specimens and sections.

The invagination of the stomodeum does not seem to take place until after the embryo has left the shell. Three pairs of branchial clefts were observed on the eighth day. The first pair, the *hyomandibular*, is developed about the time the heart begins to pulsate.

The heart has the simple form shown in fig. 21 when pulsation first begins, and the lumen is not very well marked. Its wider part indicates where the future venous end will be. Its development and separation of a ventricle will best be understood by a comparison of figs. 19–26.

The eyeballs begin to be pigmented some hours before the first specimens of a batch are hatched, and those which are hatched out later have them fully and darkly pigmented before leaving the egg.

After the blastopore has closed, the tail is developed as a *free* prolongation of the vertebral column; and as soon as it has fairly left the yolk, the first folding of the epiblast to form the continuous embryonal dorsal and ventral fin is to be seen. After the pulsation of the heart begins, the development of the tail is more rapid, and its gradual extension over the yolk will be easily followed by a comparison of the accompanying figures, up to the

point where the apex is hidden in ordinary views behind the head.

The areas where the embryonal pectoral fins are to develop are at first granular patches situated on each side about the level where the diverticulum of the liver arises. Within the area of each patch, a little later, a longitudinal ridge-like thickening of the epiblast is pushed out, which gradually extends into a fin. The ventral fins are formed in a similar manner somewhat later. The position of these fins, at first parallel to the notochord, afterwards at a considerable angle to it, will best be seen from the figures. After hatching, the bases of both pairs of fins are drawn down ventrally, and as the yolk-sac becomes absorbed, the fins lie closer to the body.

No detailed observations have been made on the development of the excretory organs and genital ducts. The vesicle near the anus which develops into the urinary bladder (*u.v.*) was first noticed on the seventh day. Its appearance at time of hatching is shown in fig. 27, with the commencement of the *Wolffian duct* (*w.d.*) leading from it.

The young fish generally begin to hatch out on the tenth day; somewhat accelerated individuals and batches on the ninth, and slightly retarded ones not till the eleventh day. Young fish of the same batch would continue to hatch out at intervals for two or three days afterwards. The young fish, for some hours after hatching, lies on its side, or more often quite on its back, but begins to right itself as the large yolk-sac is absorbed. The length of the newly hatched embryo is 3·5 millim. The mouth, which is only indicated by a slight depression in the newly hatched embryo, is well formed, and the jaws have a slight motion 24 hours afterwards, and by this time also the yolk-sac has become entirely absorbed.

Although I have been able to keep some specimens a week after hatching, further development was slight and probably abnormal, as I never succeeded in feeding any of the young fish.

Summary.

The egg of *Trachinus* is about 1·32 millim. in diameter, and contains from 20 to 30 small oil-globules, thus differing from the majority of floating fish eggs hitherto described.

In the unfertilized egg a vitelline membrane is easily distin-

guishable, but afterwards this comes in close contact with the zona radiata, and often requires the action of reagents to show it properly.

My observations appear to confirm those of Henneguy, that the invagination observed in optic section in the living egg is an inward folding of the lower layer cells of the epiblast, and that afterwards the alimentary tract is built up from this layer, together with material derived from the intermediary layer. This point cannot, however, be settled definitely without a careful examination of sections of this stage.

My observations confirm those of Ryder as to the nature and persistence of the segmentation-cavity, and in this respect pelagic teleostean eggs seem to differ from all others hitherto described.

Although the heart appears early on the fourth day, its venous end remains closely applied to the vitellus up to several days after hatching, and I have not been able to find any vascular system either in the embryo or in the vitellus up to 14 or 15 days after development begins, that is 4 or 5 days after hatching. In this respect the observations of Ryder and Kingsley and Conn agree with my own, although in non-pelagic teleostean eggs an elaborate circulatory system is developed both in the vitellus and in the embryo a considerable time before hatching.

I have nothing new to record in the later stages of development. The liver and the pancreas arise as little pouches budded off from the mesenteron; the proctodeum arises late, but is well formed at the time of hatching. The stomodeum does not appear to develop until the embryo has left the shell. The young fish usually hatch out on the tenth or eleventh day after impregnation, the early ones with little pigment on the eyes and body, the later ones with the pigment much more developed.

DESCRIPTION OF THE PLATES.

Lettering used throughout Plates.

b. c. = breathing-chamber.
h. = hypoblast.
b. = blastoderm.
s. c. = segmentation cavity.
b. r. = blastodermic rim.
k. = keel.
o. l. = optic lobes.
a. s. = auditory sacs.
k. v. = Kupffer's vesicle.
b. p. = blastopore.
h. = heart.
e. s. = embryonic shield.

g. = oil-globules.
v. = vitelline membrane.
n. z. = "nuclear zone" of Kupffer.
n. = notocord.
p. c. = pericardial sinus.
l. = liver.
z. r. = zona radiata.
y. = yolk.
p.v. = protovertebræ.
p. f. = pectoral fin.
v. f. = ventral fin.
f. b. = fore brain.
m. b. = mid brain.
h. b. = hind brain.
c. = cerebellum.
m. o. = medulla oblongata.
p. g. = pineal gland.
p. s. = pigment-spots.
m. = mesenteron.
p. = pancreas.
n. p. = nasal pit.
o. p. = invagination for eye-lens.
u. v. = urinary vesicle.
w. d. = Wolffian duct.

Figures 1–9, 10, 12, 13, 15, 16, 19, 21–24 are views looking down on egg as floating freely, and, since animal pole is downwards, represent in all cases ventral surface of the blastodisk, embryo, &c., as seen through the transparent intervening yolk-mass. All the sketches are made from the living egg, and changes that might be attributed to death or to effect of reagents are completely excluded.

In Plate III. the darker tint indicates blastodisk proper, and the pale tint shading off the intermediary layer.

In Plate IV. medium tint indicates embryonal shield and structures formed from it, embryo proper being marked by a deeper tint, whilst a light tint washed over whole marks boundary and extension of the growing blastoderm.

In Plates V. and VI. embryo proper and its parts are alone tinted; whilst in fig. 27 extent of yolk-mass is shown by a darker tint.

Plate III.

Fig. 1. Formation of first furrow in the germinal disk. ×26.
2. Appearance when outline of first two cells is formed. ×26.
2*a*. Transverse section of above, showing that the first furrow penetrates the whole thickness of the germinal disk. ×26.
3. First formation of the second furrow at right angles to the first. ×26.
4. Completion of the four-cell stage with nuclei. ×26.
5. Direction of the furrows which divide the four cells into eight. ×26.
6. Eight-cell stage after the nuclei have disappeared and the furrows commenced which divide the disk into sixteen cells. ×26.
7. Sixteen-cell stage, complete with nuclei. Outside the disk will be observed the collection of granules and free nuclei, in which the intermediary layer is developed. ×26.
8. Later segmentation-stage, in which the blastoderm consists of more than one layer of cells, and around the disk will be seen the first row of the intermediary layer cells formed by free cell-formation. ×26.
9. A little later stage, in which three rows of cells have been formed in the intermediary layer; but the outlines of the cells are not distinguishable, and the nuclei alone remain to mark the position of each cell. ×26.

PLATE IV.

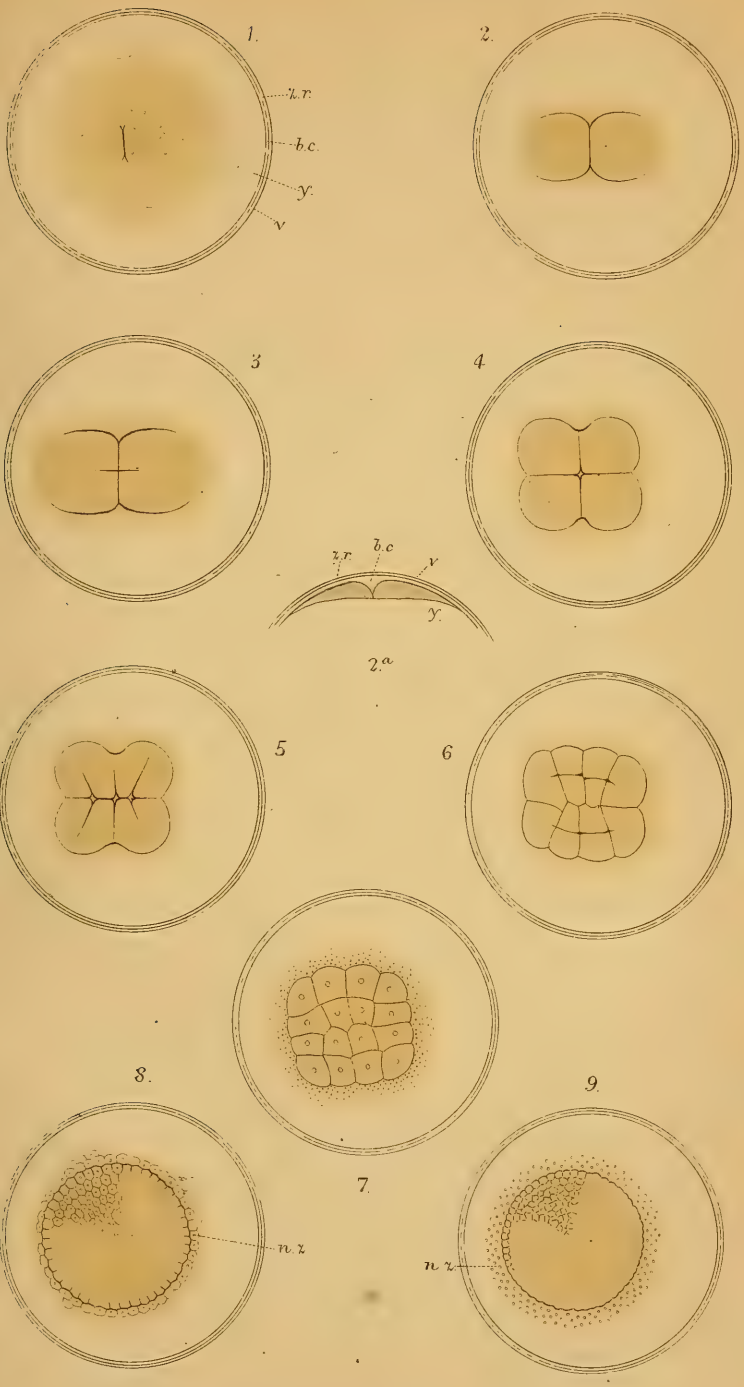
Fig. 9*a*. Side view of above, as seen floating at the surface of the water. ×26.
10. Early stage of the invagination process, as seen from beneath: (*a*) the hickened part which forms the shield in which the keel is afterwards developed. ×26.
11. Side view of above. ×26.
12. Embryonic shield well advanced, and just before the keel begins to form as a faint longitudinal streak. ×26.
13. Later stage, in which the anterior portion of the embryonic axis is defined, and the thickening for the optic lobes is seen. ×26.
(*x*) Posterior end of embryo turned upwards to eye of observer.
14. The blastoderm has here spread more than halfway over the yolk, and with it the segmentation-cavity is carried along. ×26.
15. Embryo of third day, showing thickening where the auditory sacs will be formed. ×26.
16. Embryo later on third day, showing four protovertebræ. ×26.
17. Embryo shortly before closure of blastopore. ×26.
18. Side view of part of fig. 17, showing the position of Kupffer's vesicle. ×26.

PLATE V.

Fig. 19. Embryo of fourth day, showing auditory sacs, heart, and 16 protovertebræ. ×26.
20. Side view of preceding figure. ×26.
21. Embryo of fifth day. ×26.
22. Embryo of sixth day. ×40.
23. Embryo of seventh day. ×40.
24. Embryo of eighth day. ×40.
25. Appearance of the heart on the eighth day.
26. Appearance of the heart on the tenth day.

PLATE VI.

Fig. 27. Newly hatched embryo with pigment-spots accurately marked. ×28.
28. Ventral view of above, showing position of the pectoral and ventral fins, as seen through transparent yolk-sac. ×19.
29. Embryo three days after hatching. ×28.
30. Dorsal view of above, showing change in position of the fins, and the further development of pigment. ×55.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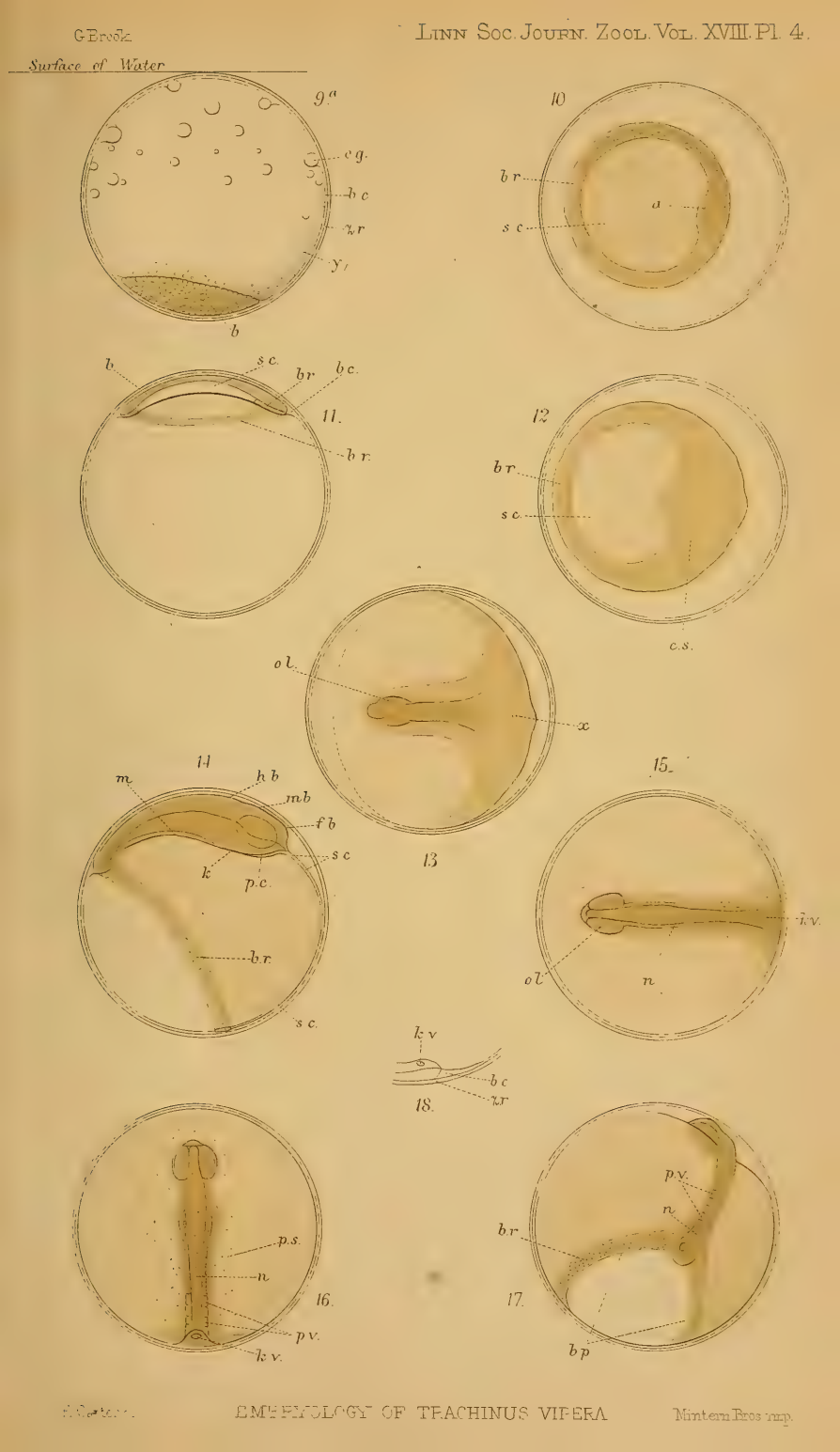
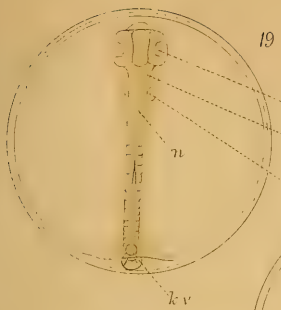


EMBRYOLOGY OF TRACHINUS VIPERA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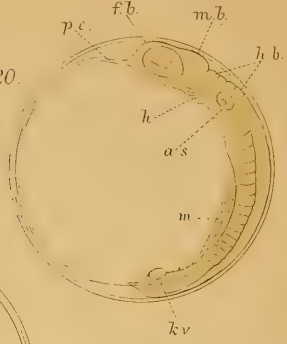
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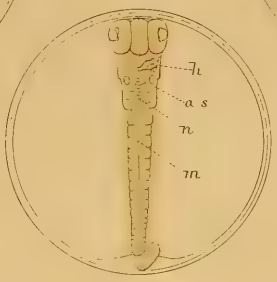
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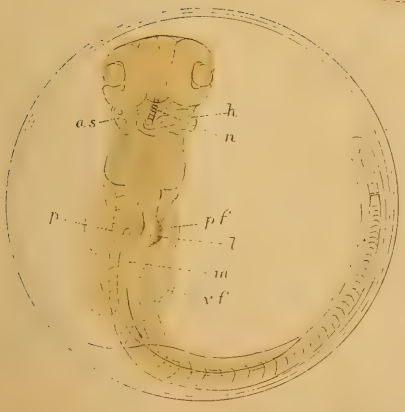
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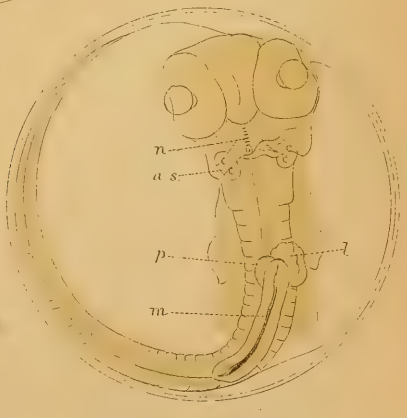
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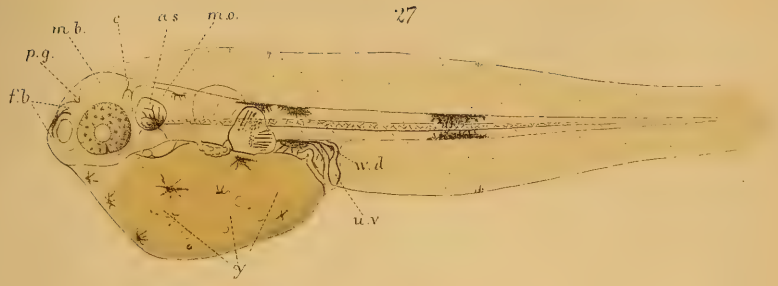

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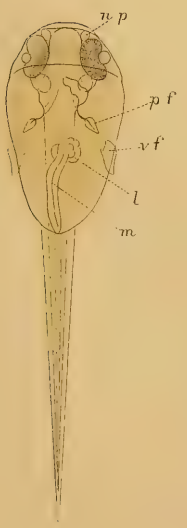

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